

USSN: 09/719,867

Page 2

Amendments to the Claims:

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims

Claim 1 (Currently amended): A process for identifying and characterizing mutations ~~leading to a selectable phenotype~~ mutations that confer resistance to a compound comprising:

- a) generating a defined set of overlapping 10 kb PCR products- PCR products, of approximately 10 kb to approximately 15 kb, containing random point mutations which encompass the complete chromosome of an organism; a wild-type strain of bacteria for which the chromosomal sequence is known;
- b) transforming pools of 12 the PCR products from step(a) corresponding to about 100 kb of the chromosome into a wild-type background; the wild-type strain and isolating the resulting strains that are resistant to a compound;
- c) isolating strains of bacteria resistant to compound;
- d) re-transforming sensitive bacteria with individual products, 10 kb, from resistant strains thereby identifying a region with one or more mutations;
- e) generating smaller PCR products, 1 kb, to further map mutation(s) responsible for phenotype; and
- f) sequencing DNA from region conferring resistance to identify the chromosomal mutation; transforming the wild-type strain with the individual PCR products from a resistant strain isolated in step (b) and isolating the resulting strains that are resistant to the compound;
- d) generating smaller PCR products of approximately 1 to approximately 4 kb which encompass the PCR product from a resistant strain identified in step (c);
- e) transforming a wild-type strain with one of the smaller PCR products from step (d) and determining whether the strain is resistant to the compound;
- f) repeating step (e) for each of the smaller PCR products until a strain resistant to the compound is found or until all of the smaller PCR products have been evaluated;

USSN: 09/719,867

Page 3

g) sequencing the smaller PCR product isolated from a strain resistant to the compound and comparing the sequence to the corresponding sequence in a wild-type strain to determine the mutation or mutations that confer resistance to a compound.

Claim 2 (cancelled)

Claim 3 (withdrawn): Mutations in *Neisseria gonorrhoeae* GyrA associated with quinolone resistance selected from: Asp90 to Glu, Ser91 to Cys, Asp95 to His, Glu161 to Gly, Glu161 to Lys, Asn65 to His, Asp80 to Gly, and Glu62 to Lys.

Claim 4 (original): The process according to Claim 1 for identifying and characterizing drug-target interactions.

Claim 5 (currently amended): A process for identifying and characterizing a mutations that confer resistance to a compound mechanism of action of an antibacterial compound comprising:

generating overlapping PCR products of approximately 10 kb to approximately 15 kb which encompass the complete chromosome from a wild-type bacteria strain for which the chromosomal sequence is known, under conditions that allow for mutation of the fragments;

allowing one or more of the generated DNA fragments-PCR products to be incorporated into the

chromosome of wild-type bacteria;

isolating bacterial strains that demonstrate resistance to a compound; and

identifying the by polymerase chain reaction amplification of DNA from bacteria under conditions that allow for mutation responsible for the resistance of the fragments;

allowing one or more of the generated DNA fragments to be incorporated into the chromosome of a bacteria by homologous recombination;

isolating the bacteria that demonstrate resistance to an antibacterial compound;

and

identifying the mutation contained in the DNA fragment.

USSN: 09/719,867

Page 4

Claim 6 (Currently amended): A process for identifying mutations that confer resistance to a compound mutations contained in the chromosome of a bacteria that results in an identifiable phenotype comprising:

- a) generating overlapping PCR products of approximately 10 kb to approximately 15 kb which encompass the complete DNA fragments by polymerase chain reaction amplification of the bacterial chromosome from a strain of corresponding to regions of the bacterial chromosome which demonstrates resistance to a compound may contain a mutation;
- b) allowing one or more of the generated DNA fragments-PCR products to be incorporated into the chromosome of a wild-type bacteria that does not display the identifiable phenotype by homologous recombination;
- c) isolating bacterial strains that demonstrate resistance to the compound; and the identifying the mutation responsible for the resistance phenotype;
repeating steps a through c until a single DNA fragment less than about 10 kilobases in length is identified as being responsible for the phenotype; and identifying the mutation contained in the DNA fragment.

Claim 7 (Cancelled)

Claim 8 (original): The process of claim 1 where the bacteria is from the group of genus *Neisseria*, *Haemophilus*, *Streptococcus*, *Staphylococcus*, or *Escherichia*.

Claim 9 (Original): The process of claim 5 or 6 where the bacteria is from the group of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, or *Escherichia coli*.

Claim 10 (Currently amended): The process of claim 5 where the antibacterial compound is a fluoroquinolone.

USSN: 09/719,867

Page 5

Claim 11 (Currently amended): The process of claim 5 where the antibacterial compound is ciprofloxacin.

Claim 12 (Currently amended): The process of claim 5 where the antibacterial compound is clinafloxacin.

Claim 13 (Currently amended): The process of claim 5 where the antibacterial compound is dihydroxydiphenylether (DHDPE).

Claim 14 (Currently amended): The process of claim 5 where the antibacterial compound is Triclosan.

Claim 15. (Cancelled).

Claim 16 (Currently amended): compound inhibits the growth or survival of the wild-type bacteria under any condition.

Claim 17 (Currently amended): The process of claims-1 ~~or~~ 2 in which the antibacterial compound inhibits the growth or survival of the wild-type bacteria in culture.

Claim 18 (Currently amended): The process of claims 1 ~~or~~ 2 in which the antibacterial compound inhibits the growth or survival of the wild-type bacteria in an animal host.

Claim 19 (Currently amended): The process of claims 1 ~~or~~ 2 in which the antibacterial compound is an inhibitor of type II topoisomerases.

Claim 20 (Currently amended): The process of claims 1 ~~or~~ 2 in which the antibacterial compound is an inhibitor of FabI.

Claim 21 (Currently amended): The process of claims-1 ~~or~~ 2 in which the antibacterial compound is an inhibitor of enzymes involved in fatty acid biosynthesis.

Claim 22 (Currently amended): The process of claim 6 in which a strain of bacteria which demonstrates resistance to a compound ~~carrying the mutation~~ was isolated from a culture that had been treated with a chemical mutagen.

Claim 23 (Currently amended): The process of claim 6 in which a strain of bacteria which demonstrates resistance to a compound ~~carrying the mutation~~ was isolated from a culture that had been treated with ultraviolet light.

USSN: 09/719,867

Page 6

Claim 24 (Currently amended): The process of claim 6 in which a strain of bacteria which demonstrate resistance to a compound carrying the mutation was isolated from a culture that in which the bacteria had been subjected to a mutagenic protocol that consisted of insertion of DNA into the chromosome of the bacteria.

Claim 25 (Withdrawn): Bacteria comprising a protein in which a contiguous stretch of 40 amino acids is at least 30% identical to residues 75 to 114 of the *Neisseria gonorrhoeae* GyrA and the residue analogous to:
62 is lysine or
63 is arginine or glutamic acid or
65 is histidine or
135 is valine or
161 is glutamic acid or lysine or glycine.

Claim 26 (Withdrawn): *Escherichia coli* strains comprising a GyrA protein in which the amino acid analogous to the *Neisseria gonorrhoeae* GyrA amino acid
62 is lysine or
63 is arginine or glutamic acid or
65 is histidine or
135 is valine or
161 is glutamic acid or lysine or glycine

Claim 27 (Withdrawn): *Neisseria gonorrhoeae* strains comprising a GyrA protein in which the amino acid residue
62 is lysine, or
63 is arginine or glutamic acid, or
65 is histidine, or
80 is alanine or glycine, or
90 is arginine or glutamic acid, or
91 is tyrosine or alanine or cysteine, or
92 is proline, or
95 is arginine or alanine or valine or tyrosine or histidine or glycine, or
114 is histidine, or
135 is valine, or
161 is glutamic acid or lysine or glycine.

Claim 28 (Withdrawn): *Neisseria gonorrhoeae* strains selected from the group consisting of NG-2707, GC318, NG-2721, NG-2711, NG-2706, NG-2717, NG-2687, GC158, NG-2690, GC219, GC291, NG-2691, NG-2720, NG-2723, GC156, NG-2698, NG-2709, NG2716, NG-2719, and NG-2712.

Claim 29 (Withdrawn): A protein comprising in which a contiguous stretch of 40 amino acids is at least 30% identical to residues 75 to 114 of the *Neisseria gonorrhoeae* GyrA and the residue analogous to:

USSN: 09/719,867

Page 7

62 is lysine or
63 is arginine or glutamic acid or
65 is histidine or
135 is valine or
161 is glutamic acid or lysine or glycine.

Claim 30 (Withdrawn): *Neisseria gonorrhoeae* GyrA protein comprising amino acid substitutions when residue

62 is lysine, or
63 is arginine or glutamic acid, or
65 is histidine, or
80 is alanine or glycine, or
90 is arginine or glutamic acid, or
91 is tyrosine or alanine or cysteine, or
92 is proline, or
95 is arginine or alanine or valine or tyrosine or histidine or glycine, or
114 is histidine, or
135 is valine, or
161 is glutamic acid or lysine or glycine.

Claim 31 (Withdrawn): Bacteria comprising a protein that is at least 30% identical to the sequence of the *Neisseria gonorrhoeae* FabI protein in which the amino acid residue corresponding to

15 is valine, or
20 is threonine, or
23 is glycine, or
25 is valine, or
51 is threonine, or
91 is threonine, or
93 is cysteine or serine, or
95 is valine, or
104 is leucine, or
105 is histidine, or
144 is valine, or
147 is histidine, or
159 is alanine, or
160 is isoleucine, or
162 is valine, or
193 is asparagine or valine, or
201 is valine, or
203 is tyrosine or valine, or
204 is serine or leucine or isoleucine or valine, or
212 is threonine or valine, or
247 is asparagine.

USSN: 09/719,867

Page 8

Claim 32 (Withdrawn): A *Neisseria gonorrhoeae* strain selected from the group consisting of NG-2669, NG-2654, NG-2651, NG-2670, NG-2660, NG-2641, NG-2639, NG-2638, NG-2640, NG-2648, NG-2657, NG-2656, NG-2653, NG-2658, NG-2663, NG-2642, NG-2671, NG-2652, NG-2661, NG-2644, NG-2667, NG-2665, NG-2655, NG-2643, NG-2666, NG-2664, NG-2647, NG-2646, NG-2650, NG-2649, NG-2645, NG-2659, NG-2662, and NG-2672.

Claim 33 (Withdrawn): An *Escherichia coli* strain comprising a FabI protein with the amino acids analagous to the ones described in claim 31 with the exception of mutations resulting in changing residue
93 to alanine or serine or cysteine or valine,
159 to threonine or,
203 to leucine.

Claim 34 (Withdrawn): A protein comprising at least 30% identical to the sequence of the *Neisseria gonorrhoeae* FabI protein in which the amino acid residue corresponding to
15 is valine, or
20 is threonine, or
23 is glycine, or
25 is valine, or
51 is threonine, or
91 is threonine, or
93 is cysteine or serine, or
95 is valine, or
104 is leucine, or
105 is histidine, or
144 is valine, or
147 is histidine, or
159 is alanine, or
160 is isoleucine, or
162 is valine, or
193 is asparagine or valine, or
201 is valine, or
203 is tyrosine or valine, or
204 is serine or leucine or isoleucine or valine, or
212 is threonine or valine, or
247 is asparagine.

Claim 35 (Withdrawn): A *Neisseria gonorrhoeae* FabI protein comprising the amino acid corresponding to residue:
15 is valine, or
20 is threonine, or
23 is glycine, or
25 is valine, or
51 is threonine, or

USSN: 09/719,867

Page 9

91 is threonine, or
93 is cysteine or serine, or
95 is valine, or
104 is leucine, or
105 is histidine, or
144 is valine, or
147 is histidine, or
159 is alanine, or
160 is isoleucine, or
162 is valine, or
193 is asparagine or valine, or
201 is valine, or
203 is tyrosine or valine, or
204 is serine or leucine or isoleucine or valine, or
212 is threonine or valine, or
247 is asparagine.

Claim 36 (Currently amended): AThe process of screening compounds for antibacterial activity comprising:

- ~~a) generating DNA fragments by polymerase chain reaction amplification of DNA from an entire genome of a bacteria under conditions that allow for mutation of the fragments;~~
- ~~allowing one or more of the generated DNA fragments to be incorporated into the chromosome of a bacteria by homologous recombination;~~
- ~~isolating the bacteria that demonstrate resistance to an antibacterial compound;~~
- ~~identifying the mutation that confers resistance to a compound using the method of Claim 1, Claim 5, or Claim 6; contained in the DNA fragment;~~
- b) contacting a strain of the bacteria containing the mutation with a
compounds; and
- c) evaluating the compounds for antibacterial activity.